Australian Government
Department of Industry, Science and Resources

National Measurement Institute

CIPM MRA

NATA
WORLD RECOGNISED ACCREDITATION

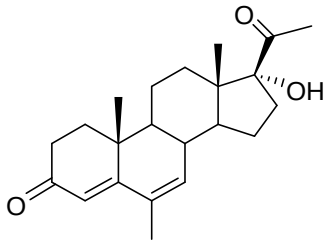
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D651: Megestrol

Report ID: D651.2024.01 (Bottled 160510)

Chemical Formula: $C_{22}H_{30}O_3$

Molecular Weight: 342.5 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
00-AV-03	3562-63-8	99.0 ± 0.8%

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: 17-Hydroxy-6-methylpregna-4,6-diene-3,20-dione

Expiration of certification: The property values are valid till 3 December 2034, ten years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: Pale yellow crystals prepared by synthesis and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 4 ºC in a closed container in a dry, dark area.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials.

The long-term stability of the compound in solution has not been examined.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with UV detection on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

Safety: Treat as a hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
17 December 2024

This report supersedes any issued prior to 17 December 2024.

NATA Accreditation No. 198 / Corporate Site No. 14214.

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognise the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in the KCDB (for details see http://www.bipm.org/kcdb/). The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by elemental microanalysis.

HPLC:	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	Methanol/water (75:25) (March 2010 and March 2015) Methanol/water (70:30) (April 2020 onwards)
	Flow Rate:	1.0 mL/min
	Detector:	PDA at 293 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.11% (6 sub samples in duplicate, March 2010)
	Re-analysis:	Mean = 98.9%, s = 0.13% (5 sub samples in duplicate, March 2015)
	Re-analysis:	Mean = 98.9%, s = 0.04% (5 sub samples in duplicate, April 2020)
	Re-analysis:	Mean = 98.9%, s = 0.04% (5 sub samples in duplicate, December 2024)
HPLC:	Column:	Waters Nova Pak C-18, 5 μm (3.9 mm × 150 mm)
	Mobile Phase:	Methanol/water (70:30)
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.12% (10 sub samples in duplicate, August 2000)
	Re-analysis:	Mean = 99.9%, s = 0.04% (3 sub samples in duplicate, March 2005)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (March 2010) Moisture content < 0.1% mass fraction (March 2015) Moisture content 0.1% mass fraction (January 2020) Moisture content < 0.1% mass fraction (December 2024)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2 % mass fraction (September 2000 and April 2005)

Spectroscopic and other characterisation data

GC-MS:	*Tris*-trimethylsilyl derivative:	
	Instrument:	HP 6890/5973
	Column:	HP Ultra 1, 17 m × 0.20 mm I.D. × 0.11 μm
	Program:	170 °C (0.5 min), 10 °C /min to 300 °C (3 min)
	Injector:	260 °C
	Split ratio:	40/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	The retention time of the tris-TMS derivative is reported with the major peaks observed in the mass spectrum. The latter are reported in mass/charge ratios and (in brackets) as peak percentage relative to the intensity of the base peak.	
	Tris-TMS (9.8 min): 558 (M+, 16), 453 (16), 231 (25), 147 (17), 73 (100) *m/z*	
ESI-MS:	Instrument:	Finnigan TSQ-700
	Operation:	Positive ion mode, direct infusion in 7.5 mM NH_4OAc, pH 4.2: MeOH (1:1)
	Scan:	Scan range *m/z* 50-600, spray voltage: 4.5 kV
	Major ions:	343 (100, $[MH]^+$) *m/z*
	Operation:	Negative ion mode, direct infusion in 7.5 mM NH_4OAc, pH 4.2: MeOH (1:1)
	Scan:	Scan range *m/z* 50-600, spray voltage: 3.0 kV.
	Major ions:	401 (100, $[M+CH_3COO]^-$), 387 (20, $[M+45]^-$), 341 (2, $[M-H]^-$) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (4:1) Single spot observed, R_f = 0.29 (5 samples)
IR:	Instrument:	Perkin-Elmer FT-IR
	Range:	4000-400 cm^{-1}, KBr disc
	Peaks:	3495, 1703, 1645, 1623, 1576, 1275, 1239, 888 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.79 (3H, s), 1.09 (3H, s), 1.84 (3H, s), 2.28 (3H, s), 5.83 (1H, s), 5.98 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (76.9 ppm)
	Spectral data:	δ 15.3, 16.4, 19.8, 20.2, 23.4, 27.8, 30.2, 33.6, 33.7, 34.1, 36.1, 37.0, 47.9, 48.9, 50.4, 89.6, 121.2, 131.3, 138.3, 164.2, 199.9, 211.1 ppm
Melting point:		200-205 °C
Microanalysis:	Found:	C = 77.2%; H = 8.6% (August 2000)
	Calculated:	C = 77.2%; H = 8.8% (Calculated for $C_{22}H_{30}O_3$)